Double-helix microscopy for wide-field 3D single-molecule fluorescence imaging

Ginni Sharma[1], Sri Rama Prasanna Pavani[2], Sean Quirin[1], and Rafael Piestun[1]

[1]*Department of Electrical, Computer and Energy Engineering, University of Colorado, Boulder, CO 80309, USA*

[2]*Department of Electrical Engineering, California Institute of Technology, Pasadena, CA 91125, USA*

ginni.sharma@colorado.edu

Abstract: We present methods to improve the localization accuracy in wide-field 3D single-molecule double-helix microscopy. We analyze the optical efficiency of the system, the fundamental limit for 3D localization, the estimation algorithms, and polarization sensitive detection.

OCIS codes: 110.0180, 110.3055, 180.6900, 100.6640.

1. Introduction

Recent advances in 3D wide-field fluorescence microscopy have demonstrated localization accuracy under 20nm in all three dimensions [1-4]. Super-resolution in the axial dimension, using single sided detection, has been achieved with three main methods that apply different principles for depth discrimination [1-4]. Astigmatic imaging [1] modifies the point spread function (PSF) for depth discrimination by introducing a cylindrical lens, the bi-plane method [2,3] uses simultaneous imaging of two planes (in-focus and out-of-focus), while the double-helix (DH) microscope modifies the PSF of the system to produce two lobes that rotate with defocus [4]. In DH microscopy, the angular orientation of the lobes encodes the axial position of a molecule, while the midpoint of the lobes represents the transverse position of the molecule. With a single DH-PSF image, the 3D position of multiple molecules can be localized with nanometer scale accuracies.

In this report, we present three fundamental improvements to the method presented in Ref. [4]. First, we engineer the 3D PSF to increase the efficiency of the DH-PSF that leads to better localization accuracy. The second improvement involves a method to extract polarization specific information using two polarization channels. The third development introduces a more accurate 3D localization estimation algorithm.

2. Fundamental limits of 3D localization accuracy in single-molecule fluorescence microscopy

The information theoretic measure of Cramer-Rao lower bound (CRB) gives the best achievable localization accuracy for a given PSF, noise, and sampling in the imaging system. Engineered PSFs provide more degrees of freedom to control localization accuracy and depth-of-field than lens based systems [5]. For instance, we demonstrate a new DH-PSF design with lower CRB for a shorter depth-of-field. We compare the fundamental limit on localization accuracy for the three methods described above (Fig. 1).

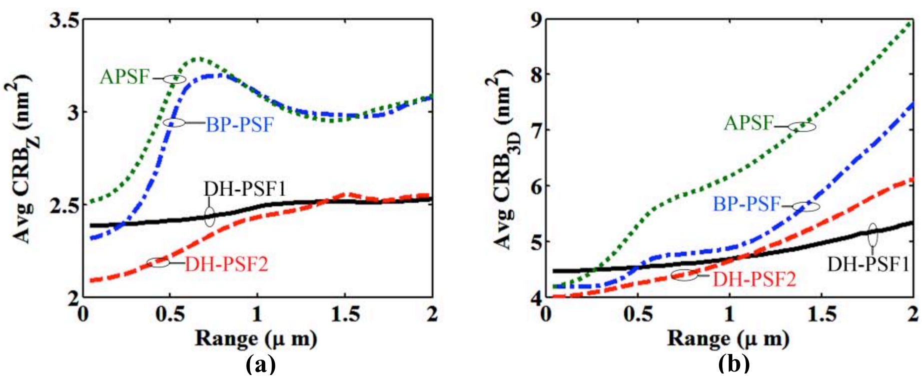

Figure 1: Comparison of the CRB for four systems: DH-PSF-1, DH-PSF-2, bi-plane (BP-PSF) and astigmatic PSF (APSF). The two plots show the variation in average CRB along the axial direction (CRB_Z) and in 3D (CRB_{3D}) with the range of measurement (depth of field). The calculation parameters are NA = 1.4, M = 100X, λ = 633 nm, pixel size = 6.3 µm x 6.3 µm and detector size = 497.7 µm x 497.7 µm. The only noise source in the system is the shot-noise which is modeled as Poisson noise with SNR = 30 for a clear aperture system (equivalent to 10113 detected photons). The DH-PSF-1 and DH-PSF-2 are generated using two different phase mask designs, the bi-plane system has two image planes 500 nm apart and the astigmatic system has two one-dimensional foci shifted by 500 nm.

The CRB plots in Fig. 1 show two different DH-PSF designs. DH-PSF1 operates on a longer depth-of-field than DH-PSF2 at the expense of 3D localization accuracy for shorter depth-of fields [5]. This design freedom is not available in lens based methods [6]. The system parameters for the astigmatic and bi-plane methods are chosen to optimize 3D localization over a depth-of-field of up to 2μm and are consistent with designs reported elsewhere. Figure 1.a represents the average CRB for axial position estimation as a function of the selected depth of field (range of the measurement). Figure 1.b shows the average 3D CRB as a function of the selected range. These plots show the benefit of using the DH-PSF to attain low CRB for axial and 3D localization over extended depths of field.

3. Polarization sensitive double-helix photoactivation-localization microscopy (PS-DH-PALM)

DH-PALM with two polarization channels is used to reveal the polarization specific characteristics of single-molecules within the intracellular structure of PtK1 cells expressing Photoactivatable Green Fluorescent Protein [7]. Up to 30% improvement in 3D localization accuracy over single channel methods is demonstrated by optimally combining the position information from the two imaging channels. This improved precision can be understood as the result of the addition of the Fisher Information from the two channels. PS-DH-PALM provides a new contrast mechanism to extract information from the sample without compromising the resolution.

4. Efficient estimator

Estimators that utilize the geometric convenience of the DHPSF, such as centroid estimation of the DH lobes to calculate the respective angle of rotation, yield rapid results for 3D position estimation [4,7]. However, these methods fail to account for the complete information capacity of each image by ignoring the other attributes of the PSF. Here we present a PSF image-matching algorithm to reduce the uncertainty of the estimation. In order to implement this algorithm we need the experimental 3D PSF as it varies continuously through focus. For this purpose, we implement a phase retrieval technique [8] on experimental calibration data sets that provide constraints on the field on discrete planes through the depth of focus (Fig. 2). As a result, the algorithm provides an estimate of a given experimental PSF based on continuous data through focus, thus using all of the information content of the image. In particular, the estimator takes into account possible aberrations of the system. This method is tested and compared with prior estimators showing significant improvements in axial localization of a fluorescent bead.

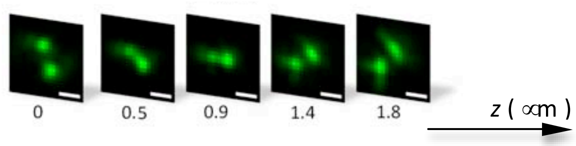

Figure 2: The experimental images used for the implementation of an efficient estimator using a DH-PSF. The images are obtained by moving a 40nm wide fluorescent bead in the axial dimension. The algorithm interpolates these images using phase retrieval. The estimator takes into account the information content of the entire experimental PSF image including possible aberrations. Scale bar: 1∝m

5. Conclusion

We demonstrate methods to enhance single-molecule double-helix microscopy, including polarization-sensitive detection to achieve a new contrast mechanism, DH-PSF design to allow control of the tradeoff localization accuracy / depth-of-field, and estimation algorithms to improve 3D localization accuracy.

6. References

[1] B. Huang, W. Wang, M.Bates, X. Zhuang, "Three-Dimensional Super-Resolution Imaging by Stochastic Optical Reconstruction Microscopy," Science **319**, 810–813 (2008).
[2] S. Ram, P. Prabhat, J. Chao, E. S. Ward, and R. J. Ober, "High accuracy 3D quantum dot tracking with multifocal plane microscopy for the study of fast intracellular dynamics in live cells," Biophys. J. **95**, 6025–6043 (2008).
[3] M. F. Juette, T. J. Gould, M. D. Lessard, M. J. Mlodzianoski, B. S. Nagpure, B. T. Bennett, S. T. Hess, and J. Bewersdorf, "Three-dimensional sub-100 nm resolution fluorescence microscopy of thick samples," Nat. Meth. **5,** 527–529 (2008).
[4] S. R. P. Pavani, M. A. Thompson, J. S. Biteen, S. J. Lord, N. Liu, R. J. Tweig, R. Piestun, and W. E. Moerner, "Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function," PNAS, **106**, 2995-2999 (2009).
[5] G. Sharma, S. R. P. Pavani, and R. Piestun, "Fundamental limits on 3D particle localization in photon-limited microscopy," to be submitted.
[6] C. v. Middendorff, A. Egner, C. Giesler, S. W. Hell, and A. Schonle, "Isotropic 3D Nanoscopy based on single emitter switching," Opt. Exp. **16**, 20774-20788 (2008).
[7] S. R. P. Pavani, J. G. DeLuca, and R. Piestun, "Polarization sensitive, three-dimensional, single-molecule imaging of cells with a double-helix system," Opt. Express **17**, 19644-19655 (2009).
[8] L. J. Allen and M. P. Oxley, "Phase retrieval from series of images obtained by defocus variation", Opt. Comm. **199**, 65-75 (2001)